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Lipopolysaccharide structure impacts the entry kinetics of bacterial outer membrane vesicles into host cells

O'Donoghue, Eloise; Sirisaengtaksin, Natalie; Browning, Douglas; Bielska, Ewa; Hadis, Mohammed; Fernandez-Trillo, Francisco; Alderwick, Luke; Jabbari, Sara; Krachler, Anne Marie

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1	Supporting Information for
2	Lipopolysaccharide structure impacts the entry kinetics of bacterial outer
3	membrane vesicles into host cells
4	Eloise J O'Donoghue ¹ , Natalie Sirisaengtaksin ² , Douglas F. Browning ¹ , Ewa Bielska ¹ ,
5	Mohammed Hadis ³ , Francisco Fernandez-Trillo ³ , Luke Alderwick ¹ , Sara Jabbari ^{1,4} and Anne
6	Marie Krachler ^{2,*}
7	
8	¹ Institute of Microbiology and Infection, School of Biosciences, University of Birmingham,
9	Edgbaston, B15 2TT Birmingham, UK
10	² Department of Microbiology and Molecular Genetics, University of Texas McGovern Medical
11	School at Houston, Houston, TX, 77030, USA.
12	³ Institute of Microbiology and Infection, School of Chemistry, University of Birmingham,
13	Edgbaston, B15 2TT Birmingham, UK
14	⁴ School of Mathematics, University of Birmingham, Edgbaston, B15 2TT, Birmingham, UK
15	*Correspondence to: Anne Marie Krachler (anne.marie.krachler@uth.tmc.edu)
16	
17	This file contains:
18	Supporting Materials and Methods
19	Supporting Figures S1-S6
20	

22 SUPPORTING MATERIALS AND METHODS

23 Nanoparticle tracking analysis

After purification, OMV samples were diluted 1x10⁻⁶ in filtered sterile PBS. Particle diameter and concentration were measured using the Nanosight LM10 particle tracking analysis, with a minimum of 100 tracks per sample, performed in triplicate. Camera shutter 1495 and gain of 450 were used, and size distribution scatter plots were created using GraphPad Prism. Size distribution was analysed using analysis of variance (ANOVA) with a Brown Forsythe test for equal variance.

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31 Measurement of ζ-potential as an indicator of OMV surface charge

32 700 µl of OMV preparations were analysed using a Zeta Sizer (Malvern Instruments) and data

33 from an average of 30 readings/sample were acquired at 37 °C and means were plotted.

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35 Visualization of outer membrane vesicles by Transmission Electron Microscopy

36 10µl of isolated outer membrane vesicles in sterile deionized distilled water were added to 400-37 mesh copper grids, and negatively stained with 4% uranyl acetate for 2 min. Samples were then 38 observed using a Jeol 1200Ex transmission electron microscope (Birmingham Electron 39 Microscopy Facility) with an acceleration of 75kV.

41 SUPPORTING FIGURES



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54 55 Figure S2. Rates of uptake/dismantling and concentration dependency of uptake kinetics 56 for OMVs. (A) CCF2-AM loaded Hela cells exposed to EHEC OMVs carrying ClyA-Bla (red), 57 or empty vector (grey) at an MOI of 1000 for 3 h. Rate of uptake over time was extracted from 58 data in Figure 2A and data shown are means ± stdev (n=3). (B) FRET change upon exposure of 59 Hela cells to EHEC OMVs carrying ClyA-Bla (reporting on exposure to OMV surface to 60 cytoplasm) or Bla-ClyA (reporting on exposure of luminal cargo to cytoplasm). (C) Hela cells 61 were exposed to EHEC or K12 ClyA-Bla OMVs at an MOI of 1000 for 3 hours. Rates of uptake 62 over time were extracted from data in Figure 3A and are means \pm stdev (n=3). (D) Experiments 63 were repeated as above but using different OMV concentrations (0-20 µg/ml of protein, corresponding to an MOI of 0- 2000), and maximum rates (D) and efficiency of uptake (E) 64 65 determined as described above. Data are means \pm stdev (n=3).



Figure S3. Uptake for OMVs from serotypes O157, O42 and O16 with or without O antigen. CCF2-AM loaded RKO intestinal epithelial cells were exposed to OMVs from EHEC O157 (A), EAEC O42 (B), and K12 O16 (C), with O antigen (red) and without O antigen (blue), at an MOI of 1000 for 3 hours. FRET changes (blue/green fluorescence, A-C) and efficiency of uptake (total change over three hours, D) are shown as means ± stdev (n=3).

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- 74





Figure S4. Rates of uptake for OMVs from serotypes O157, O42 and O16 with or without O 77

78 antigen. CCF2-AM loaded Hela cells were exposed to OMVs from EHEC O157 (A), EAEC O42 79 (B), and K12 O16 (C), with O antigen (red) and without O antigen (blue), at an MOI of 1000 for

80 3 hours. Polynomials were fitted to each data set using the cubic spline function csaps in Matlab.

81 Numerical estimates of the gradients of the resulting polynomials were determined using the

82 gradient function. Data shown are means \pm stdev (n=3).

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Figure S5. Effect of blebbistatin and dynasore on uptake of OMVs. Hela cells were either left untreated or pre-treated 80 uM Dynasore for dynamin inhibition (grey), or 20 uM blebbistatin for macropinocytosis inhibition (orange) for 1h at 37 °C and exposed to ClyA-Bla OMVs isolated from EHEC (A, B), EAEC (C, D), or K12 (E, F) at an MOI of 1000 for 3 hours. The FRET signal (ratio of blue:green fluorescence) over time was plotted as mean ± stdev (n=3).



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Figure S6. Effect of pharmacological treatments on OMV uptake. Hela cells were either left untreated or pre-treated with 5 ug/ml papain (lilac), 1 ug/ml chlorpromazine (pink), 5mM methyl- β -cyclodextrin (light green) or 1µg/ml filipin (turquoise) and exposed to ClyA-Bla OMVs isolated from EHEC (A, B), EAEC (C, D), or K12 (E, F) at an MOI of 1000 for 3 hours. The FRET signal (ratio of blue:green fluorescence) over time was plotted as means ± stdev (n=3).